the design of the device. Also important to consider is the problem of evaporation with the very small volumes that are frequently involved with the operations. Where there is an interest in doing a quantitative analysis of an operation involving kinetics, it is important that the solvent volume remain substantially constant, so that the concentrations of the reactants are not changing due to decreasing volume. To this end, methods are required to minimize evaporation. Additionally, particularly where long incubation times and/or long reaction times are involved, there is an interest in preventing contamination. A further concern is unintentional pressurization, during closing of a microstructure vessel, which could prematurely move the liquid from a reservoir into a channel.

A2

On page 1, please replace the paragraph starting on line 30 with the following:

In response to these concerns, lids have been used to seal the ports of microfluidic devices. Lids may provide sealing through the pressure of their weight, by providing adhesion, using various forms of latches or clasps, or by fitting snugly around a part of the microfluidic device and held by friction.

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On page 2, please replace the paragraph starting on line 1 with the following:

There is an interest in developing devices and methods to substantially diminish the undesired events that may occur due to open ports of a microfluidic device, where the devices are effective and can be readily fabricated and methods readily performed.

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On page 2, please replace the paragraph starting on line 6 with the following:

U.S. Patent no. 5,443,890 and references cited therein describe leakage-proof sealing of microfluidic devices. WO 99/43432 describes microfluidic devices and systems incorporating cover layers. U.S. Patent no. 5,545,280 describes applying adhesive to protrusions on a substrate.

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On page 2, please replace the paragraph starting on line 26 with the following:

Fig. 1 is a plan view of a device with collars around microstructures; Fig. 1a is a cross-sectional view of the device of Fig. 1 along the partial phantom line 1a-1a;

A6

Fig. 2 is a plan view of the device of Fig. 1, with the collars removed to provide details of the individual units; Fig. 2a is a diagrammatic exploded view of the units of Fig. 2;

Fig. 3 is a side elevation cross-sectional view of a reservoir microstructure of a unit with a cover.

On page 3, please replace the paragraph starting on line 4 with the following:

The following examples are offered by way of illustration of the present invention, not limitation. Microfluidic devices are provided for manipulation of small volumes, where the devices comprise a substrate, usually an organic substrate in which there are channels and reservoirs, where the reservoirs have a raised collar above the planar surface of the substrate. A bottom film, including a rigid substrate, is adhered to and encloses the channels and the bottoms of the reservoirs. The reservoirs can be sealed on the top side using a film, which seals to the upper surface of the collar.

On page 3, please replace the paragraph starting on line 18 with the following:

The sealing cover or lid will be a film, which forms a seal about the collar to at least substantially inhibit fluid flow from the reservoir. The cover will provide for sealing interaction with the collar upper surface, as a result of a compliant surface contacting the collar or an adhesive surface adhering to the upper surface of the collar, particularly an adhesive surface, which is removable. Contact will usually be minimal or not at all between the sealing cover or lid and the planar surface. The forces providing the sealing may be gravity, adhesive forces, or mechanical forces. For compliant surfaces, such as elastomeric films, skin-surface (closed-cell) foams, soft films, pressure would be applied. The pressure may be a result of a weighted backing, or a latching or gripping device for holding the film against the collars. The pressure may further be a result of a vacuum chuck which holds the film in position and can release the film, as appropriate, etc. The film may be stretched across the collars, held in position by clasps at he periphery of the substrate, a sealing pliable band around the periphery, a vacuum chuck, etc. A continuous sealing film may be used, which may be unrolled from a reel as the devices are moved in a continuous manner, for example, on a wheel or moving belt. The films may be natural rubber, polyisoprene, ethylene-propylene elastomers, polyurethane foams,

polydimethylsiloxane, etc. The films may be thin or thick, so long as they have a minimum dimension, which provides for their sealing of the collars. Generally, the films will be at least about 50μm in thickness. Alternatively, films may be used, which have a thin adherent layer, which will adhere to the surface of the collar and after the film has fulfilled its function, the adhesive may be removed. Useful adhesives include pressure sensitive adhesives, such as ethylene-containing polymers, urethane polymers, butyl rubber, butadiene-acrylonitrile polymers, butadiene-acrylonitrile-isoprene polymers, and the like. See, for example, U.S. Patent no. 5,908,695 and references cited therein.

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On page 4, please replace the paragraph starting on line 9 with the following:

The substrate will generally have a thickness of at least about 20μm, more usually at least about 40μm, and not more than about 0.5cm, usually not more than about 0.25cm. The width of the substrate will be determined by the number of units to be accommodated and may be as small as about 2mm and up to about 6cm or more. The dimension in the other direction will generally be at least about 0.5cm and not more than about 20cm, usually not more than about 10cm. The substrate may be a flexible film or relatively inflexible solid, where the microstructures, such as reservoirs and channels, may be provided by embossing, molding, machining, etc. The collars may be formed at the same time using the same process, although more expensive processes may be used, such as photolithography or laser ablation. In this case, the collar regions would be protected while the substrate was eroded. The channel dimensions will generally be in the range of about 0.1μm to 1mm deep and about 0.5μm to 500μm wide, where the cross-section will generally be $0.1 \mu m^2$ to about $0.25 mm^2$. The channel lengths will vary widely depending on the operation for which the channel is to be used, generally being in the range of about 20nl to 1μl. The reservoirs may be cylindrically shaped or conically shaped, particularly inverted cones, where the diameter of the port will be from about 1.5 to 25 times, usually 1.5 to 15 times, the diameter of the bottom of the reservoir, where the reservoir connects to the channel.

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On page 4, please replace the paragraph starting on line 28 with the following:

Whether the microfeatures are left open will depend upon (i) whether a supporting film and/or an enclosing film is provided and/or (ii) whether the device is produced by embossing a film or by molding. The supporting film will generally be at least about 40μm and not more than about 5mm thick. The film used to enclose the channels and the bottom of the reservoirs will generally have a thickness in the range of about 10μm to 2mm, more usually in the range of about 20μm to 1mm. The selected thickness is primarily one of convenience and assurance of good sealing and the manner in which the devices will be used to accommodate instrumentation. Therefore, the ranges are not critical.

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On page 5, please replace the paragraph starting on line 20 with the following:

As indicated, the substrate may be a flexible film or inflexible solid, so the method of fabrication will vary with the nature of the substrate. For embossing, at least two films will be used, where the films may be drawn from rolls, one film embossed ant the other film adhered to the embossed film to provide a physical support. The individual units may be scored, so as to be capable of being used separately, or the roll of devices retained intact. See, for example, application serial no. PCT/98/21869. Where the devices are fabricated individually, they will usually be molded, using conventional molding techniques. The substrates and accompanying film will generally be plastic, particularly organic polymers, where the polymers include additional polymers, such as polyethers, polyesters, polyamides, polyimides, etc. Desirably, the polymers will have low fluorescence inherently or can be made to have low fluorescence by additives or bleaching. The underlying enclosing film will then be adhered to a substrate by any convenient means, such as thermal bonding , adhesives, etc. The literature has many examples of adhering such films, see, for example, U.S. Patent nos. 4,558,333; and 5,500,071.

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On page 7, please replace the paragraph starting on line 28 with the following:

The subject invention provides many advantages in enclosing, usually reversibly, small reservoirs or other microstructure. The subject collar structure has a small contact area, which serves to concentrate the force produced by whatever means of application of the lid onto a much smaller area, as compared to a cover which bonds to the entire

A12

surface of the device. A reduction in differential pressures created during application of the lid is achieved. Where the upper surface is flat, without areas in relief, a conformal lid comes down in such a way that it will usually first make contact with a large ring around the area to be sealed. The air trapped within this ring is pressurized into the volume to be sealed. By contrast, with the subject structure the lid makes contact before it reaches the device main surface, and thereby avoiding the problem of trapping large volumes of air in the sealed area. In addition, for lid attachment mechanisms like gravity, friction and mechanical clips, where the force is not directly related to the contact area, the subject method increases the local pressure with which the lid is attached to the part. This increased pressure generally improves the seal and improves the proximity of conformal lids. This improved seal can enable the use of a weighted lid to produce an airtight seal without requiring a large mass or an extremely conformable lid material. Where the seal is substantially air tight, the lid will act to resist or prevent fluid flow. Capillary stop junctions may be prone to failure by condensation or other mechanism, in which case the sealed lid provides a backup mechanism. Also, the sealed lid can easily counteract relatively strong fluidic forces, such as surface tension. Yet another advantage of the collar is that the effective leakage path (surface path) between two adjacent wells is increased. This increases the resistance to arcing due to high differential voltages in two adjacent wells. In fact, multiple concentric collars would be beneficial in further reducing this problem.
a12 Finally, during application and removal of a lid, there is a potential for liquids to wick between the lid and the surface of the device and microstructures. The short distance between the device and the lid can result in very strong capillary pressures. In the subject invention the lid is only in close proximity to the collar surface, so that fluid can only wick along that surface. By avoiding continuous ridges between different microstructures, movement of liquid between the microstructures can be obviated.

On page 8, please replace the paragraph starting on line 24 with the following:

a13 All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications mentioned in this specification are herein incorporated

by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

a13

In the Claims:

Please cancel claim 1.

Please replace claims 2-5, and 12 with the following re-written claims:

2. (Amended) A microfluidic device according to Claim 3, wherein said collar has its inner surface aligned with the inner surface of said opening and a thickness of from about 0.05 to 0.5 mm thick extending away from said inner surface.

3. (Amended) A microfluidic device comprising:

a substrate having a planar surface having at least one microstructure formed thereon, a plurality of openings in said surface of microstructures and at least a portion of said openings surrounded by a collar in relief, and wherein said collar is covered with a lid of a conformable material.

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4. (Amended) A microfluidic device comprising:

a substrate having a planar surface having at least one microstructure formed thereon, a plurality of openings in said surface of microstructures and at least a portion of said openings surrounded by a collar in relief, and wherein said collar is covered with a lid with an adhesive coating.

5. (Amended) A microfluidic device according to Claim 3 produced by plastic molding.

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12. (Amended) In a method employing a microfluidic device comprising introducing small volumes into microstructures, where the volumes comprise volatile solvents, the improvement which comprises: introducing said small volumes into a device according to Claims 3 or 4; and applying a compliant or adhesive lid to each of said collars.

Please add new claims 15-16 as follows:

15. (New) A microfluidic device according to Claim 4, wherein said collar has its inner surface aligned with the inner surface of said opening and a thickness of from about 0.05 to 0.5 mm thick extending away from said inner surface.

a16 16. (New) A microfluidic device according to Claim 4 produced by plastic molding.

In the Drawings:

Applicants respectfully propose a correction to Fig. 1, as shown in the enclosed red-lined drawing page and as described below.

Fig. 1 is described on page 2, line 27 to depict a line 1a-1a.